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# Chromosome-Specific Telomere Length in Women with Breast Cancer: Their Relationship to Chemotherapy and Acquired Psychoneurological Symptoms

Areej Alhareeri

*Virginia Commonwealth University*

# Chromosome-Specific Telomere Length in Women with Breast Cancer: Their Relationship to Chemotherapy and Acquired Psychoneurological Symptoms

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Human and Molecular Genetics at Virginia Commonwealth University

By

Areej Abdulaziz Alhareeri
B.S.c. (Applied Medical Sciences), King Saud University, 2005
Saudi Arabia

Director: Colleen Jackson-Cook, Ph.D.
Professor
Department of pathology

Virginia Commonwealth University
Richmond, VA
April, 2013

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# Abstract

CHROMOSOME-SPECIFIC TELOMERE LENGTH IN WOMEN WITH BREAST CANCER: THEIR RELATIONSHIP TO CHEMOTHRAPY AND ACQUIRED PSYCHONEUROLOGICAL SYMPTOMPS

Areej Abdulaziz Alhareeri, B.S.

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Breast cancer (BC) is one of the most common diagnosed malignancies in females. Although 90% of early diagnosed women are expected to survive for at least 5 years, their quality of life is adversely affected by a cluster of symptoms which we collectively named “psychoneurological symptoms’’ (PN). Given that acquired telomere attrition has been speculated to be a causal factor in chronic diseases and the lack in

the literature of mechanisms giving rise to PN symptoms, this study was performed to assess telomere length using a chromosome-specific telomere assay before receiving chemotherapy and at the first chemotherapy. We showed significant telomere attrition on the short arm of chromosome 9. In addition, we showed a negative correlation between telomere length and depression. Furthermore, we evaluated several variables as predictors of the change in telomere length and showed that chemotherapy was predictive of shortened telomere length. Taken together, one can speculate that shortened telomeres could result in epigenetic alterations in the genes juxtaposed to the telomeric region, giving rise to the development and persistence of PN symptoms. Knowledge gained from this study will offer hope for the development of therapeutic interventions that could prevent undesirable side effects and ensure a better quality of life for patients with BC.

# Chapter 1

## Introduction

In 2009 the Nobel Prize in Physiology or Medicine was jointly awarded to Elizabeth Blackburn, Carol Greider and Jack Szostak “for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase”. The structure of the telomere was first recognized by Hermann Muller and Barbara McClintock through their studies in Drosophila (Muller, 1938) and maize (McClintok, 1941). Muller concluded that a special structure at the end of the chromosome was required for its integrity and first coined the term ‘telomere’. Three years later, McClintock (1941) proposed that telomeres stabilize chromosome ends and prevent them from being recognized as DNA double strand breaks.

As a result of intensive research that has been completed since these pioneering studies, much is now known about telomeres. Telomeres can now be more precisely described as being comprised of non-coding tandem arrays of a “TTAGGG” DNA sequence that are located at the terminal ends of all vertebrate chromosomes including those of humans (Moyzis, et al., 1988). A G-rich single stranded 3’ overhang is present at the end of human telomeres and is thought to be important for telomere function (Makarov et al., 1997, Wright et al., 1997). This single stranded 3’ overhang folds back on itself forming a large loop structure called a telomere loop, or T-loop that has a shape similar to that of a paper clip. The telomere is stabilized by a six-protein complex called shelterin, which includes Telomeric Repeat binding Factor 1 and 2

(TRF1 andTRF2), Protection of Telomeres 1(POT1), TRF1 and TRF2 Interacting Nuclear protein 2(TIN2), the human ortholog of the yeast Repressor/Activator protein 1(Rap1) and TPP1. Shelterin components specifically localize to the telomere due to the recognition of TTAGGG repeats by three of its components: TRF1 and TRF2 recognize the duplex part of telomeres and bind to it, whereas POT1 recognizes the single stranded repeat sequence in the 3' overhang localized within the T-loop structure (specifically within the "displacement" or D-loop). TIN2, TPP1, Rap1 and POT1 are recruited to the telomere by TRF1 and TRF2 (Palm & de Lange, 2008).

In 1961, Leonard Hayflick and Paul Moorhead discovered that cells cultured *in vitro* undergo a limited number of divisions, a phenomenon which is now known as replicative senescence or the "Hayflick limit", and suggested the existence of a cellular counting mechanism (Hayflick & Moorhead, 1961). Furthermore, they observed the presence of two broad classes of cells, normal mortal cell strains and immortal cancer cell lines (reviewed in Hayflick, 1998). Their discovery challenged a central dogma of the time that supported the belief that cultured cells could divide indefinitely *in vitro*, with any culture failures being attributable simply to a lack of technical expertise.

By combining the knowledge that the properties of DNA replication prevent cells from fully replicating the ends of linear chromosomes (Watson, 1972) with the observation that normal cells have a limited capability to replicate, Olovnikov proposed his theory of marginotomy (1973). It has been reported that he developed this hypothesis while waiting for a subway train in Moscow. As he heard the train coming, he imagined the train, and specifically the engine, being the DNA polymerase and the track

being the DNA. The engine (DNA polymerase) would not be able to replicate the first segment of DNA (the track) because it lay exactly underneath the engine. It seemed unlikely that with each cell division a DNA segment containing important genes was lost. Therefore, Olovnikov reasoned that the repeated non-coding telomeric nucleotide sequences act as a buffer to protect gene coding sequences. He correctly speculated that with each round of cell division a portion of the telomere "buffer" would be lost, and that the length of the telomeric "buffer" could be important for determining a cell's ability to proliferate (reviewed in Hayflick, 1998; Greider, 1998).

In testing the hypothesis that telomere length was related to the proliferative capacity of cultured cells, Harley, et al (1990) found that the mean telomere length was decreased by two to three kilobase pairs (kbp) during the entire life time of multiple strains of cultured human fibroblasts. In contrast immortal cell lines, identified by Hayflick, appeared to avoid telomere shortening with serial passages *in vitro* (reviewed in Hayflick, 1998). Insight as to the reason for this apparent paradox was gained by Greider and Blackburn (1985), who discovered enzymatic activity in Tetrahynema cell extracts that compensated for the incomplete replication of telomere ends. They subsequently purified the enzyme and found that it was a riboucleoprotein (RNP) that was comprised of an RNA component, as well as a protein component. Thus, they had discovered a new type of DNA polymerase, which they named "telomerase" (Greider & Blackburn, 1987). Moreover, they discovered that the RNA component contained the template required for the addition of nucleotide repeats onto telomeres (Greider and Blackburn, 1989). Although all of these findings were in Tetrahymena, it is now known

that telomerase provides the main mechanism by which telomeres are elongated in eukaryotes.

Human telomerase is composed of a reverse transcriptase protein (TERT) consisting of 1132 amino acids encoded by the hTERT gene, located on the short arm of chromosome 5 (5p15.33). Additionally, it's composed of telomerase RNA containing 451 nucleotides encoded by the telomerase RNA gene hTERC, which is located on the long arm of chromosome 3 (3q21-q28) (reviewed in Aubert & Lansdorp, 2008). Telomerase activity is present in germ cells, stem cells, cancer cells and a subset of somatic cells (such as active fibroblasts). However, most somatic cells lack telomerase activity, leading them to having a limited life span. In cells lacking telomerase, telomeres shorten with each round of cell division, due to the end replication problem. Gradual telomere shortening that accompanies cellular proliferation eventually results in excessive telomere shortening, leading to the activation of a DNA damage response (DDR) at the telomere end, which, in turn, gives rise to permanent growth arrest (senescence) (reviewed in Cesare & Reddel, 2008).

In addition to averting the end replication problem, telomeres also function to prevent chromosomal ends from being distinguished as DNA double strand breaks; thereby avoiding the activation of DNA damage signaling pathways and genome instability (de Lange, 2009). Ataxia telangiectasia mutated (ATM) kinase pathway and ataxia telangiectasia and Rad3 related (ATR) kinase pathway are activated in mammalian cells in response to DNA double strand breaks in order to arrest the cell cycle and induce apoptosis (programmed cell death). Moreover, homology directed

repair (HDR) and non-homologous end joining (NHEJ) repair pathways could also be activated to ensure that cells continue dividing with an intact genome. Telomeres prevent the effect of these four different pathways by sheltrin. The TRF2 subunit of sheltrin is responsible for ensuring that the ATM kinase signaling pathway and NHEJ repair pathway are not activated by T-loop formation. On the other hand, POT1 subunit is responsible for repressing ATR kinase signaling pathway by preventing RPA, the single stranded DNA binding protein, from binding to the telomeric single stranded DNA. Both TRF2 and POT1 block the activation of HDR pathway. Failure to do so causes cell cycle arrest under the direction of: a) ATM and/or ATR b) chromosome end-to-end fusion mediated by NHEJ or c) sequence exchange by HDR involving two telomeres or a telomere and another part of the genome.

Another problem solved by telomeres is avoiding the loss of valuable genetic information, which could occur as a result of a cell's inability to fully replicate the chromatin at the very end of the chromosome. This is prevented by the presence of telomere length maintenance mechanism through telomerase (de Lange, 2009). Telomere length can also be maintained through a recombination based telomerase-independent mechanism termed alternative lengthening of telomeres (ALT) (Xu et al., 2012).

Telomere attrition is one of the well-known cell intrinsic events associated with normal cellular aging (Mayer et al., 2006). More importantly, telomere attrition and dysfunction have been shown to be a causal factor in the acquisition of many age-related diseases, including, but not limited to atherosclerosis (Bentos et al., 2004);

myocardial infarction (Brouilette et al., 2003); Alzheimer's dementia (Panossian et al., 2003) and heart failure (reviewed in Oeseburg et al., 2010). Several lifestyle factors such as smoking, consumption of unhealthy diet, obesity and lack of exercise can accelerate telomere shortening (reviewed in Shammas, 2011). Telomere shortening can also occur as a consequence of oxidative damage. Because of their high guanine content, telomeres are particularly susceptible to oxidative damage that induces single and double DNA strand breaks. Investigators have shown that accumulated single strand breaks due to oxidative damage are less efficiently repaired at telomeres than any other location in the genome (Shen et al., 2009). Furthermore, interchromosomal fusion mediated by excessive telomere shortening can induce genomic instability (de Lange, 2005), which, in turn, may contribute to the development of cancer (Meeker, 2006). Cancer is a term given for a broad group of various diseases where there is uncontrolled cell growth. The progression from a normal cell to a cancer cell occurs through a number of events in which the cell acquires the so-called "hallmarks of cancer" (Hanahan & Weinberg, 2000). Immortality is considered as one of the hallmarks of cancer cells. Replicative telomere attrition is considered one of several regulatory mechanisms that blocks cell immortality (reviewed in Xu et al., 2012). Replicative senescence occurs in a cell after about fifty passages and involves permanent p53-mediated cell cycle arrest in Gap 1 (G1) phase of the cell cycle (reviewed in Raynaud et al., 2008). However, a DNA damage checkpoint deficient cell can continue dividing through p53 or pRb inactivation, resulting in extensive telomere attrition. As a result, telomeres lose their protective function allowing frequent chromosomal fusions and rearrangements, which lead to massive genomic instability, the latter of which can be

lethal for the cell. Cell death is bypassed and immortality is achieved by telomere length maintenance through the up-regulation of telomerase in 85% of human tumors (Raynaud et al., 2008). In the remaining 15% of human tumors, telomere length is maintained by ALT (reviewed in Cesare & Reddel, 2008). Several investigations have shown that telomeres are markedly shorter in invasive cancers of the breast, as well as its pre-invasive counterparts, despite the presence of telomerase activity (Meeker et al., 2004).

Breast cancer forms in tissues of the breast. The two main types of breast cancer are ductal carcinoma (forms in tubes that carry milk to the nipple) and lobular carcinoma (forms in glands that produce milk). Breast cancer may be invasive, meaning that it forms in the ducts or lobules and spreads to other tissues of the breast. Alternatively, it can be non-invasive, meaning it does not spread to other breast tissues. Estrogen receptor positive cancer refers to breast cancer in which the cells have an increased sensitivity to estrogen leading to cancer tumor growth. On the other hand, HER2- positive breast cancer has an amplification (too many copies) of the HER2 gene, which causes the tumor to grow faster and the cancer to be more aggressive. The National Cancer Institute estimated the diagnosis of 226,870 new cases of female breast cancer and the occurrence of 39,510 deaths in females with breast cancer in 2012 (most recent estimation). Therefore, breast cancer is considered one of the most common malignancies in women, accounting for about 1 in 3 cancers diagnosed among females in the United States (DeSantis et al., 2011). However, breast cancer is diagnosed in most cases at an early stage (stage I or II). Therefore, 90% of early-

diagnosed women are expected to survive for at least 5 years (Jemal et al., 2009). Patients with breast cancer experience a number of symptoms that tend to cluster with each other leading to long-term effects that adversely impact their quality of life (QOL) (Dodd et al., 2010). These symptoms, which may manifest from the breast cancer treatment and/or the cancer itself, include depression, anxiety (Badger et al., 2007), cognitive dysfunction, sleep disturbances, pain and fatigue (Kim et al., 2012). In our study, we are collectively naming these symptoms “psychoneurological (PN) symptoms”.

One-reason patients may present with a number of symptoms, rather than a single symptom, is that these symptom “clusters” may share a common biological mechanism (Miaskowski & Aouizerat, 2007). Several studies have been conducted in order to understand the biological mechanism leading to the development of PN symptoms. Symptoms of cancer/cancer treatment are thought to be due, at least in part, to cytokine-induced sickness behavior (Cleeland et al., 2003). Cytokines are present in increased levels in patients with cancer (Meyers et al., 2005) and can be produced from tumor tissues (Sasaki et al., 2001) or in response to chemotherapy (Pusztai et al., 2004). Although cytokine-induced sickness behavior is considered a reasonable mechanism leading to symptom clustering in patients with cancer, it can’t account for all the clusters reported to date. This is because several investigators have reported different numbers of symptoms in a cluster, as well as different types of symptoms within a cluster (Miaskowski and Aouizerat, 2003). Several investigators have shown an association between each of the PN symptoms and the activation of inflammation

markers, particularly proinflamatory cytokines (reviewed in Kim et al., 2012). However, these studies were limited because they focused on single symptoms and single markers of inflammation. Moreover, they haven't clearly explained the mechanism leading to the development and persistence of PN symptoms, nor have they given rise to innovative therapeutic approaches.

Recently, several different mechanisms have been hypothesized to explain the association between the development and persistence of PN symptoms and inflammation (reviewed in Ahles & Saykin, 2007). Meyers et al. (2005) reported a higher than expected incidence of some PN symptoms, as well as proinflammatory cytokines, prior to the initiation of chemotherapy. This finding suggests that inflammation may be activated by the cancer itself, with the symptoms being worsened by the chemotherapy. These findings are of interest because they suggest the need for examining PN symptoms associated with chemotherapy within the broader context of biological processes associated with the development of cancer; one of which is telomere shortening. Acquired telomere attrition has also been speculated to be a causal factor in the acquisition of many chronic diseases (Epel et al., 2004). Factors that accelerate the rate of telomere shortening include, but are not limited to, chemotherapy (Li et al., 2012) and oxidative damage (von Zglinicki, 2002). In fact, in a study by Blasiak et al. (2004) higher levels of cellular oxidative damage were observed in women with breast cancer when compared to control individuals. Furthermore, a study on leukocytes obtained from patients with early stage breast cancer showed a decreased telomere length at five and nine months following chemotherapy when compared to baseline values (Schroder

et al., 2001). However, information regarding PN symptoms was not integrated into that study, since the main focus of these investigators was to determine the effect of stem cell transplantation following chemotherapy in the evaluated group of patients.

In addition to DNA/chromosomal damage, telomere shortening could contribute to PN symptoms by influencing gene expression, since telomeric heterochromatin is known to regulate the expression of adjacent subtelomeric genes, which is a phenomenon termed "telomere position effect" (TPE) (Wright & Shay, 1992 ; Ning et al., 2003). Given all of the above findings, we opted to study the contributory role of telomere shortening in the development and persistence of PN symptoms by measuring telomere length in women having breast cancer who received treatment with chemotherapy.

The gold standard method for measuring telomere length is the terminal restriction fragment (TRF) technique. Quantitative Polymerase Chain Reaction (PCR)-based techniques have also been used in measuring telomere length. However, both techniques are limited because they do not provide information on telomere length at individual chromosome ends, but instead provide data of average genomic telomere length. Moreover, both methods do not allow the recognition of specific chromosomes with short telomeres and/or dysfunctional telomeres. Given the limitations of these techniques, we chose to measure telomere length using peptide nucleic acid – fluorescence in situ hybridization (PNA-FISH), which was developed by Lansdorp et al. (1996). This high-resolution technique not only allows the quantification of chromosome-

specific telomere length, but also provides information about consistent patterns that arise in specific chromosomal regions as a result of breast cancer or it's treatments.

The data collected from this study will allow us to test our **hypothesis that chemotherapy contributes to chromatin breakage leading to telomere shortening.** One can speculate that telomeric shortening could result in an epigenetic alteration in the genes juxtaposed to the telomeric region, giving rise to the development and persistence of PN symptoms. Toward this end we propose the following aims:

**Aim 1: To assess chromosome-specific telomere length in cultured lymphocytes of women receiving chemotherapy following a diagnosis of breast cancer.**

**Aim 2: To determine the correlation between telomere length changes and PN symptoms.**

**Aim 3: To investigate the correlation between telomere length changes and micronuclei (MN) frequency.**

# Chapter 2

## Materials and Methods

### Ascertainment of Samples

A total of 25 women with early stage (I to IIIA) breast cancer were ascertained through the Massy Cancer Center (MCC) in Virginia Commonwealth University and its multiple collaborative sites, including Hematology Oncology Associates of Fredericksburg, Inc., Bon Secours Richmond Health System, Peninsula Cancer Institute, and Rappahannock General Hospital. To identify potential study participants, a member of the research team attended the weekly interdisciplinary Breast Health meetings at Virginia Commonwealth University Health System (VCUHS). From the list of patients presented at the meeting, those persons meeting the study inclusion criteria were identified. The study coordinator then consulted with the attending oncologist and with his/her approval approached the patient regarding her desires to participate in the study. The offsite research nurses followed similar procedures for identifying potential participants at his/her respective site. After providing their informed consent (VCU IRB # HM 13194), all participants in this study completed questionnaires and cognitive testing via a computerized system at time point one (also referred to as “baseline”, which was prior to chemotherapy) and time point two (also referred to as “mid-chemo which was at the fourth chemotherapy treatment). During these baseline and mid-chemo visits, blood samples (in sodium heparin tube) were collected by venipuncture or an existing access device and transported to our laboratory in a biohazard container. The lab staff was

blinded to the PN symptom status of the study participants at the time of sample processing or evaluation.

**Cell culture, Harvest and Slide Preparation**

Upon receiving the blood samples, lymphocytes were isolated using Histopaque-1077 (Sigma). Duplicate stimulated lymphocyte cultures were established and harvested using standard procedures [RPMI 1640 media, supplemented with 15% Fetal bovine serum (FBS) and phytohemaglutinin (PHA)]. Twenty minutes prior to harvest, colcemid (final concentration of 0.1 μg/ml) was added in order to limit microtubule formation by inactivating spindle fiber formation, which arrested the cells in metaphase. Seventy-two hours after culture initiation, the cultures were harvested as previously described (Leach and Jackson Cook, 2001) using standard protocol techniques, including a 20 minute incubation in hypotonic solution (0.075 M KCl), a ten minute incubation (room temperature) in fixative (3:1 methanol: acitic acid solution) and two additional washes in fixative. In order to reduce cytoplasm formation, which can compromise probe hybridization, slides were made using a Thermotron, which provided a constant temperature (23°C) and humidity (48 ±1°C). A phase contrast/bright field microscope was used to ensure that the quality of the slide preparation was optimal for the FISH analysis (i.e. enough metaphases present that lacked cytoplasm, had good morphology, and were well spread). The slides were then placed on a hot plate at 60°C for an hour, followed by aging at room temperature for 1-2 weeks. Alternatively, after the hot plate incubation, the room temperature aging was induced by soaking the slide in a 2xSSC solution for ten minutes prior to the FISH protocol.

**Chromosome-Specific Telomere FISH**

The telomere length of metaphase chromosomes was assessed using a telomere-specific FITC-labeled peptide nucleic acid (PNA) probe, following the manufacturer's protocol (DakoCytomation, Denmark). Briefly, target slides were soaked in cold fixative (3:1 metanol: acitic acid) for one hour. Following air-drying, the slides were rinsed in 1xTBS (Triss-Buffered Saline, PH 7.5) for two minutes, fixed in 3.7% formaldehyde in 1xTBS for two minutes, and then rinsed twice in 1xTBS for five minutes each. The slides were then immersed in a pre-treatment solution containing proteinase K for ten minutes and rinsed twice in 1xTBS for five minutes each. Following dehydration in an ethanol series (70%, 85% and 100%) for two minutes each, the slides were air-dried. After drying, 22 μl of FITC $(CCCTAA)_3$ labeled PNA probe was added to each full slide and a coverslip added. Following the co-denaturation of the probe and metaphase chromosomes in a thermocycler at 80°C for three minutes, the slides were hybridized in a dry hybridization chamber at room temperature for two hours. Unbound and excess probe was then removed by washing in a rinse solution (provided by the manufacturer) at room temperature for two minutes, as well as a second wash solution (provided by the manufacture) at 65°C for five minutes. After washing, the slides were dehydrated in a series of cold ethanol solutions (70%, 85% and 100%) for two minutes each. Finally, the slides were air-dried and counterstained with 24 μl of a 5:1 DAPI II /propidium iodide (Abott) solution.

## Telomere Image Analysis

A total of ten metaphases for each individual were analyzed using software on an Applied Imaging Cytovision system (the Comparative Genomic Hybridization (CGH) function) as previously described (Leach et al., 2004). Briefly, for each metaphase analyzed, three images were captured using a CCD camera. The first image was captured using a DAPI filter to allow for subsequent karyotyping and identification of the chromosomes based on their reverse DAPI banding pattern. The second image was captured using a FITC filter, which allowed for the visualization of the telomeric probe and is termed by the software as the "test image". The third image allowed for the visualization of the chromosomal bodies based on their propidium iodide stain and is termed a "reference image". Karyograms were prepared for each metaphase spread, followed by the designation of the centromere and central axis for each homolog. For each telomere, the software compared the fluorescence intensity from the "test image" to the fluorescence intensity of the propidium iodide stain in the chromosomal body from the "reference image" to generate a ratio profile for each chromosome. For each study participant the intensity values were averaged over the twenty homologs from the ten metaphase spreads scored. Overlapping chromosomes at the telomere region were omitted from the analysis. A representative metaphase is shown in Figure 1.

## Statistical Analysis

The total number of females with early stage breast cancer enrolled in this study was 25. However, one subject was excluded from the analyses due to the lack of

metaphase yield from the blood specimen at mid-chemo. For the remaining participants, a paired t- test was used to compare the mean telomere intensity values between baseline and post-chemo. Pearson correlation was used to examine the correlation between age and telomere length, as well as between PN symptoms and chromosome specific telomere intensity values. In order to determine if there was change in the telomere intensity value of each chromosomal arm (p arm and q arm) from baseline to mid- chemo within and across all study participants, the percent change in telomere intensity was obtained using the following formula (for each woman):

$$\frac{\text{Mid-chemo telomere intensity value - Baseline telomere intensity value}}{\text{Baseline telomere intensity value}}$$

A mixed effect model was used to screen multiple variables in this study as predictors of the change in telomere length from baseline to mid-chemo. A mixed effect model is considered a statistical model that incorporates both fixed effect parameters (which are the different variables being tested in this study) and random effects (which are the subjects being tested). For all other tests, a p-value of $\leq 0.05$ was considered significant. All analyses were performed using JMP software, version 10. Figures were generated using R statistical software.

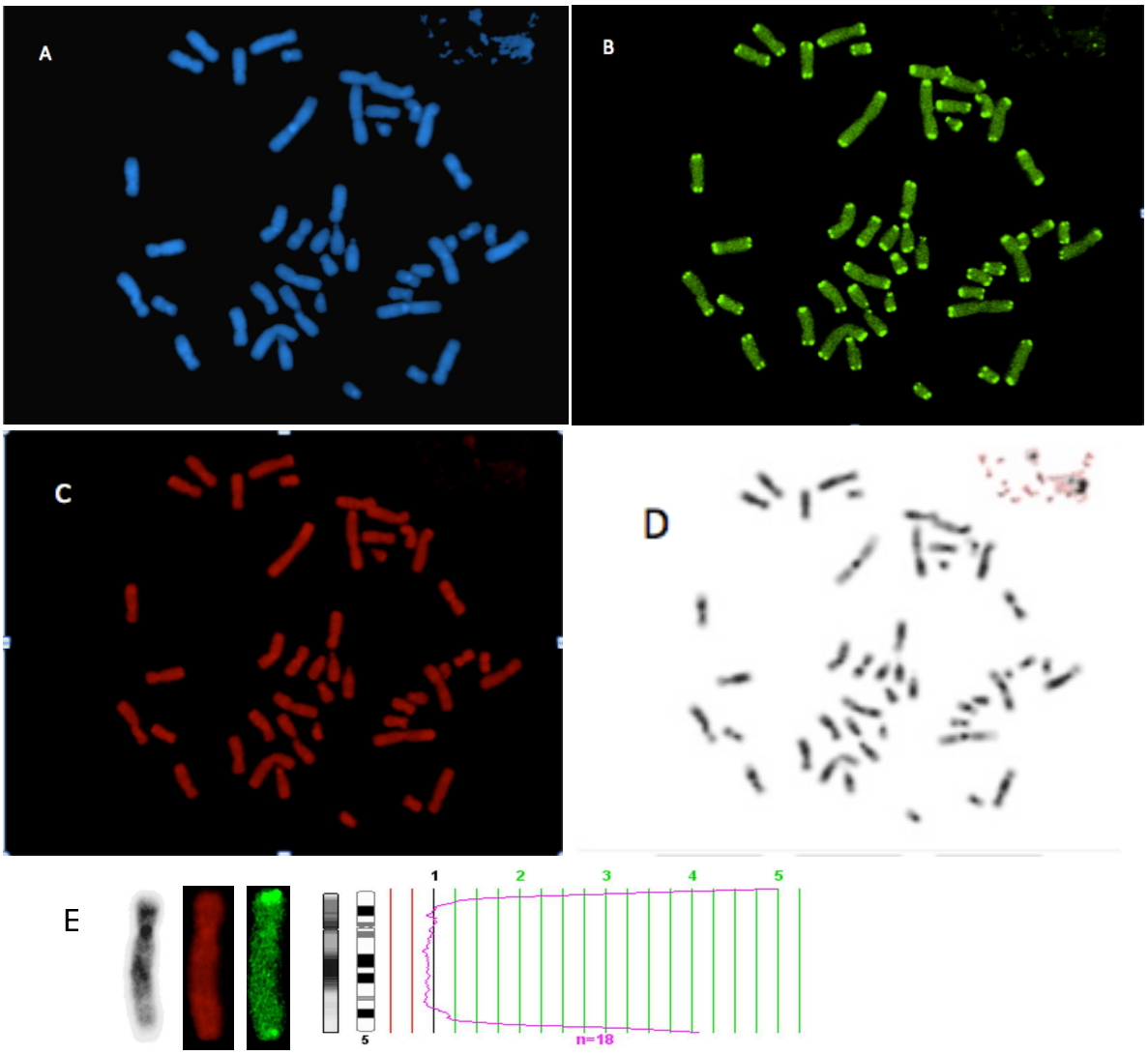


**Figure 1: A representative image of chromosome-specific telomere FISH.** For each individual, 10 metaphases were analyzed and for each metaphase 3 images were captured. (A) Shows a metaphase spread stained with DAPI, which allows for the identification of chromosomes based on reverse DAPI bands. (B) Shows a "test image" captured using a FITC filter, which allows the visualization of the telomere probe. (C) Represents the "reference image" showing the same metaphase in a PI stain. (D) The metaphase as it appears with reverse DAPI bands. (E) Chromosome as it appears in the DAPI, FITC labeled and PI image. A representative ratio profile of chromosome 5 from this cell is shown. For this chromosome the short arm telomere (top of chromosome) had a larger fluorescence intensity value (5.0) than the long arm telomere (value of 4.13).

# Chapter 3

## Results

### Sample Distribution

The 25 study participants ranged in age from 28 to 69 years old, with an average age of 49.84 and a median age of 50 years old. The distribution of the study participant's age is shown in Figure 2.

### Telomere length at baseline and mid-chemo

The mean telomere length was estimated for each individual by averaging the telomere fluorescent intensity of all chromosomes. Potential correlation between telomere length at baseline and age were determined (Table 1). As expected, based on previous studies showing that telomere length is inversely correlated with age in the general population, a negative correlation between the participants' age and their average telomere length was observed (r= -0.394, p-value=0.0564), as shown in Figure 3.

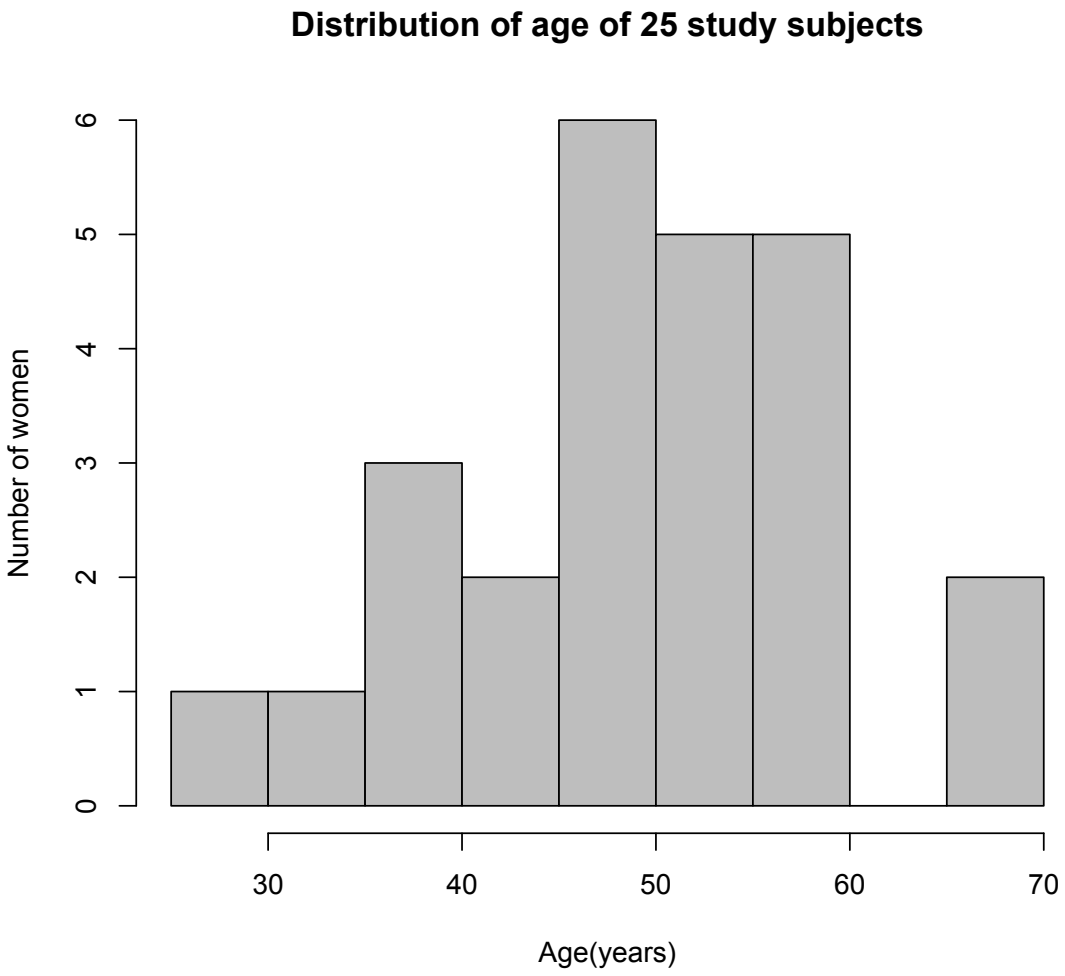


**Figure 2: Age distribution of 25 subjects.** The age range of the study participants (in 5 year brackets) is shown on the X axis, with the number of women in each age group being shown on the Y axis.

**Table 1: Age and mean telomere length of the 25 study subjects**

| Subject | Age | Mean Telomere Length |
|---|---|---|
| 2001 | 56 | 4.640 |
| 2002 | 49 | 3.149 |
| 2005 | 51 | 4.006 |
| 2006 | 41 | 5.111 |
| 2008 | 38 | 7.700 |
| 2009 | 40 | 4.372 |
| 2014 | 47 | 4.533 |
| 2017 | 48 | 2.397 |
| 2021 | 58 | 2.271 |
| 2022 | 28 | 6.550 |
| 2024 | 35 | 2.687 |
| 2027 | 60 | 2.538 |
| 2041 | 47 | 2.219 |
| 2042 | 69 | 3.157 |
| 2043 | 44 | 5.122 |
| 2044 | 55 | 5.717 |
| 2051 | 52 | 2.494 |
| 2060 | 53 | 4.840 |
| 2061 | 50 | 2.660 |
| 2062 | 66 | 2.288 |
| 2063 | 50 | 2.478 |
| 2065 | 59 | 3.725 |
| 2067 | 58 | 6.913 |
| 2070 | 53 | 6.070 |
| 2071 | 39 | 4.640 |

- Mean telomere length values are the averages of the fluorescent intensities of the 92 telomeres present in each woman's genome.

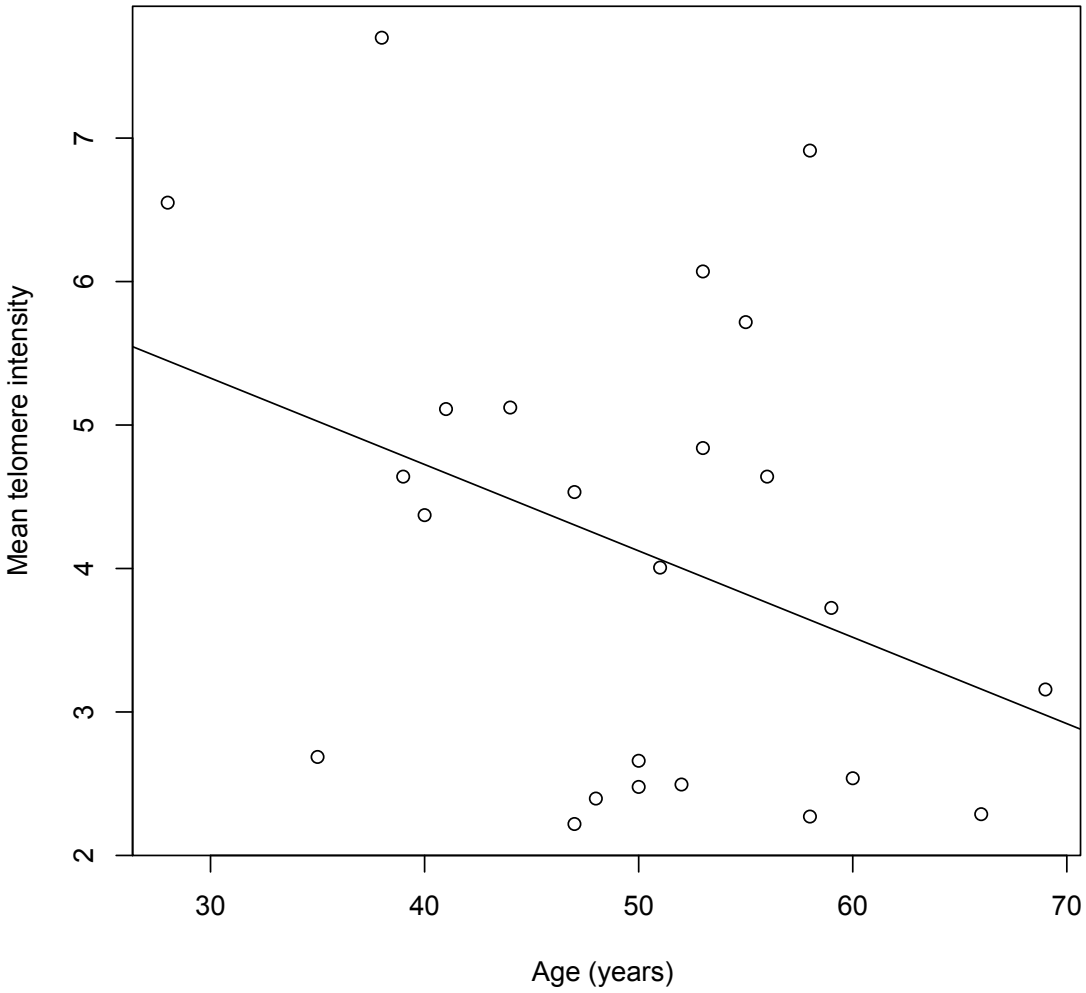


**Figure 3: Pearson's correlation between age and mean telomere intensity at baseline.** Each circle (o) represents a study subject. Mean telomere intensity at baseline was negatively correlated with age, with this trend approaching significance (r= -0.394, p-value=0.0564).

**Change of telomere length from baseline to mid-chemo**

In order to determine if there was change in the telomere intensity value of each chromosomal arm (p arm and q arm) from baseline to mid-chemo within and across all study participants, the percent change in telomere intensity values were obtained. Negative value reflected a decrease in telomere length at the mid-chemo compared to baseline time point; a zero value reflected no change in the telomere length; and a positive value reflected an increase in the telomere length at the mid-chemo time point. The percent change values for each chromosomal telomere (short arm and long arm) and each study participant are represented by a heat map (Figure 4). The frequency of telomeres showing attrition was higher than the frequency of telomeres showing increases in length as indicated by the heat map. In addition to calculating percent changes, we compared telomere fluorescent intensities of the short and long arm of the 23 chromosomes (1-22 and X) between baseline and mid-chemo using a paired t-test. A summary of the mean, standard deviation, mean of the difference and p-values are shown in Table 2. The majority of chromosomal arms did not show a significant difference between baseline and mid-chemo values. However, the short arm of chromosome 9 was observed to be significantly shorter in the mid-chemo specimens (p-value=0.038). In agreement with the t-test results, a significant change in telomere length of the short arm of chromosome 9 from baseline to mid-chemo was also detected using a mixed effect model analysis (p-value=0.038). In addition, both the t-test and the mixed effect model showed marginal significance for decreases in the telomere lengths

of the long arm of chromosomes 9, 11, 12, 15 and 18 (t-test p-value = 0.085, 0.080, 0.065, 0.089, 0.051, respectively).

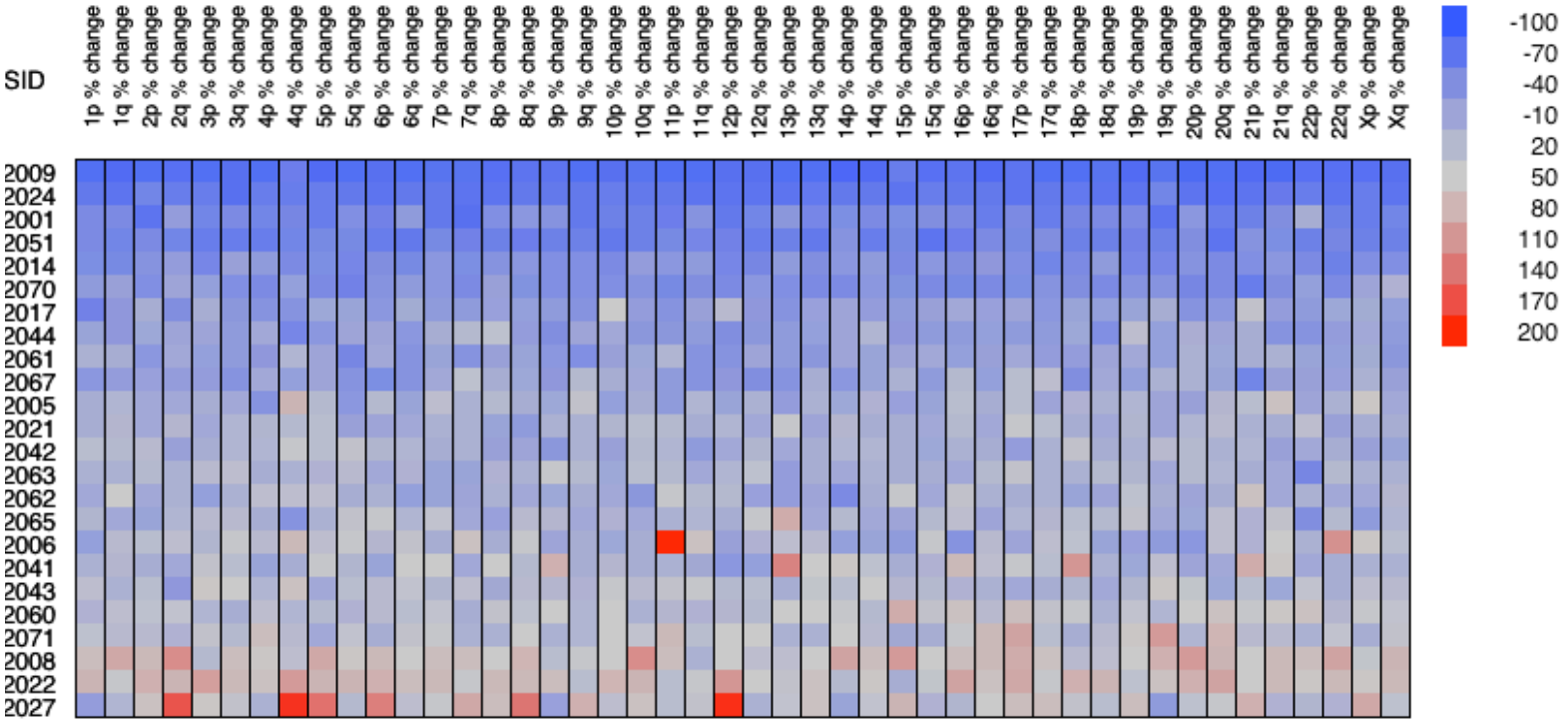


**Figure 4: Heat map representation of percent changes in telomere intensity values from baseline to mid-chemo.** Each column represents a chromosomal arm and each row represents a study subject. The legend shows the percent change for the mid-chemo specimen, with decreases in telomere length being shown in blue tones and increases in telomere length being shown in red tones.

**Table 2: A comparison of telomere intensity values between baseline and mid-chemo specimens in 24 study participants.**

| Chromosome | Short arm | | | | | | Long arm | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | Mid-Chemo | | | | Baseline | | Mid-Chemo | | | |
| | Mean | SD | Mean | SD | Mean of the difference | p-value | Mean | SD | Mean | SD | Mean of the difference | p-value |
| 1 | 3.306 | 1.301 | 2.803 | 1.427 | -0.502 | 0.137 | 4.452 | 1.862 | 3.885 | 1.679 | -0.567 | 0.254 |
| 2 | 4.460 | 2.007 | 3.871 | 1.746 | -0.588 | 0.227 | 3.257 | 1.357 | 3.052 | 1.614 | -0.205 | 0.602 |
| 3 | 5.295 | 2.080 | 4.675 | 1.900 | -0.620 | 0.243 | 4.244 | 1.981 | 3.601 | 1.703 | -0.642 | 0.195 |
| 4 | 3.778 | 1.731 | 3.148 | 1.604 | -0.629 | 0.156 | 4.677 | 1.950 | 4.440 | 1.896 | -0.237 | 0.654 |
| 5 | 4.315 | 1.790 | 3.945 | 1.803 | -0.370 | 0.474 | 4.016 | 1.910 | 3.350 | 1.808 | -0.665 | 0.162 |
| 6 | 4.681 | 2.142 | 3.948 | 1.767 | -0.732 | 0.186 | 4.338 | 1.878 | 3.668 | 1.696 | -0.670 | 0.165 |
| 7 | 3.601 | 1.750 | 3.306 | 1.619 | -0.378 | 0.356 | 4.330 | 1.977 | 3.633 | 1.693 | -0.697 | 0.192 |
| 8 | 4.486 | 2.023 | 3.932 | 1.691 | -0.554 | 0.246 | 3.379 | 1.392 | 3.190 | 1.737 | -0.189 | 0.636 |
| 9 | 4.820 | 2.125 | 3.846 | 1.655 | -0.973 | 0.038* | 3.130 | 1.428 | 2.564 | 1.264 | -0.566 | 0.085 |
| 10 | 4.590 | 1.944 | 3.978 | 1.727 | -0.612 | 0.234 | 3.840 | 1.782 | 3.368 | 1.670 | -0.472 | 0.285 |
| 11 | 3.903 | 1.543 | 3.695 | 2.206 | -0.207 | 0.701 | 4.437 | 2.143 | 3.655 | 1.746 | -0.782 | 0.080 |
| 12 | 4.073 | 1.728 | 3.403 | 1.707 | -0.670 | 0.189 | 4.112 | 1.959 | 3.254 | 1.483 | -0.857 | 0.065 |
| 13 | 4.051 | 1.599 | 3.425 | 1.262 | -0.625 | 0.147 | 4.523 | 2.126 | 3.768 | 1.710 | -0.755 | 0.139 |
| 14 | 4.061 | 1.773 | 3.424 | 1.776 | -0.637 | 0.175 | 3.944 | 1.705 | 3.442 | 1.539 | -0.502 | 0.249 |
| 15 | 3.909 | 1.574 | 3.432 | 1.689 | -0.476 | 0.222 | 4.515 | 2.046 | 3.715 | 1.750 | -0.799 | 0.089 |
| 16 | 3.367 | 1.647 | 2.950 | 1.270 | -0.417 | 0.286 | 3.608 | 1.556 | 3.197 | 1.657 | -0.411 | 0.335 |
| 17 | 3.335 | 1.346 | 3.190 | 1.555 | -0.145 | 0.726 | 3.615 | 1.772 | 3.128 | 1.667 | -0.487 | 0.219 |
| 18 | 4.473 | 2.022 | 3.892 | 1.786 | -0.581 | 0.199 | 4.700 | 2.078 | 3.787 | 1.695 | -0.913 | 0.051 |
| 19 | 3.425 | 1.435 | 3.036 | 1.359 | -0.388 | 0.327 | 3.958 | 1.583 | 3.393 | 1.637 | -0.564 | 0.226 |
| 20 | 4.168 | 1.928 | 3.618 | 1.739 | -0.550 | 0.249 | 3.508 | 1.601 | 3.015 | 1.628 | -0.492 | 0.301 |
| 21 | 4.317 | 1.930 | 3.838 | 1.622 | -0.478 | 0.367 | 3.714 | 1.615 | 3.368 | 1.588 | -0.346 | 0.416 |
| 22 | 4.242 | 1.673 | 3.563 | 1.607 | -0.679 | 0.118 | 3.453 | 1.478 | 3.040 | 1.760 | -0.412 | 0.318 |
| X | 4.602 | 2.056 | 4.119 | 1.824 | -0.482 | 0.317 | 4.129 | 1.797 | 3.601 | 1.712 | -0.528 | 0.262 |

- The p-values indicated are obtained from a t-test.
- An asterisk is indicative of a significant p-value.

**Association between chromosome-specific telomere length and psychoneurological symptoms**

To gain insight into the relationship between telomere length and each of the seven psychoneurological symptoms evaluated in this study, we performed correlation analyses between the score change of each of the symptoms and the percent change in telomere intensity values from baseline to mid-chemo for all 92 chromosomal arms. The score change was calculated by subtracting the symptom score at baseline from the symptom score mid-chemo. Interestingly, there was a significant positive correlation between depression and attrition for the telomeres localized to 3p, 3q, 4p, 5q, 6p, 6q, 10p, 11q, 12p, 12q, 13p, 13q, 14q, 15q, 18p, 18q and 19p (Table 3). Furthermore, even with the small sample size in this study, a marginally significant ($0.05 < \text{p-value} \leq 0.1$) positive correlation was observed between the symptom of depression and attrition of telomeres localized to 1p, 2p, 7q, 8p, 9q, 16q, 17q, 19q, 20p and Xq (Table 4).

In contrast to the correlation between depression and telomere length, no significant associations were observed between telomere intensity values and the single PN symptoms of pain, fatigue, anxiety, stress and sleep disturbance. The range of coefficient correlations and p-values for each symptom evaluated is shown in Table 5.

**Table 3: Significant correlations between chromosome-specific telomere length and depression**

| Chromosomal arm | Correlation coefficient ( r ) | P-value |
|---|---|---|
| 3p | 0.567 | 0.004 |
| 3q | 0.491 | 0.015 |
| 4p | 0.440 | 0.031 |
| 5q | 0.532 | 0.007 |
| 6p | 0.494 | 0.014 |
| 6q | 0.475 | 0.019 |
| 10p | 0.450 | 0.028 |
| 11q | 0.536 | 0.007 |
| 12p | 0.499 | 0.013 |
| 12q | 0.503 | 0.012 |
| 13p | 0.410 | 0.047 |
| 13q | 0.443 | 0.030 |
| 14q | 0.508 | 0.011 |
| 15q | 0.422 | 0.040 |
| 18p | 0.467 | 0.021 |
| 18q | 0.435 | 0.034 |
| 19p | 0.584 | 0.003 |

**Table 4: Marginally significant correlations between telomere-specific telomere length and depression**

| Chromosomal arm | Correlation Coefficient ( r ) | P-value |
|---|---|---|
| 1p | 0.403 | 0.051 |
| 2p | 0.399 | 0.053 |
| 7q | 0.363 | 0.081 |
| 8p | 0.371 | 0.074 |
| 9q | 0.385 | 0.063 |
| 16q | 0.400 | 0.053 |
| 17q | 0.385 | 0.063 |
| 19q | 0.381 | 0.066 |
| 20p | 0.362 | 0.082 |
| Xq | 0.352 | 0.091 |

- Marginally significant means $0.05 < \text{p-value} \leq 0.1$

**Table 5: Range of correlation coefficients and p-values of the non-significant correlations between telomere length and individual psychoneurological symptoms**

| Psychoneurological symptom | Correlation coefficient ( r ) | P-value |
|---|---|---|
| Pain | -0.274 to 0.203 | 0.194 to 0.984 |
| Fatigue | -0.388 to 0.197 | 0.149 to 0.967 |
| Anxiety | 0.034 to 0.386 | 0.058 to 0.871 |
| Stress | 0.047 to 0.380 | 0.066 to 0.824 |
| Sleep disturbance | -0.320 to -0.002 | 0.127 to 0.992 |

- The correlation coefficients and p-values show the ranges obtained from the assessment of correlations between each symptom and each chromosomal arm (total of 46 comparisons per symptom).

**Non-symptom factors influencing telomere length**

In addition to the assessment of individual PN symptoms, we also investigated the relationship (and possible inter-relationship) between telomere length change and other variables measured in this study, including (but not limited to) type of chemotherapy used in treatment, HER2 status, ER status, and demographic variables such as the education level. Screening of these variables, using mixed effect model analyses, revealed stress, cognitive functions and chemotherapy as significant predictors of changes in telomere length for a subset of specific chromosomal arms (Table 6). To tease apart the cognitive component(s) potentially influencing telomere length, each of these components were individually evaluated. The individual tests showed the memory domain, psychomotor speed domain, reaction time domain, complex attention domain, processing speed domain, verbal memory domain and visual memory domain to all be significant predictors of the change in telomere length (Table 7). On the other hand, none of the tumor marker factors or demographic variables appeared to be contributory to the change in telomere length (Table 8).

## Table 6: P-values of the factors predicting the change in telomere length

| Chromosome | Cognitive function | Parameter estimate | Stress | Parameter estimate | Chemotherapy |
|---|---|---|---|---|---|
| 1p | | | 0.030* | 0.062 | 0.066 |
| 1q | | | 0.030* | 0.076 | 0.074 |
| 2p | 0.035* | -0.017 | 0.076 | 0.069 | |
| 2q | | | 0.059 | 0.057 | |
| 4p | | | 0.025* | 0.075 | |
| 4q | | | 0.025* | 0.083 | 0.063 |
| 5p | | | 0.077 | 0.060 | 0.035* |
| 5q | | | 0.028* | 0.084 | |
| 6p | 0.022* | -0.018 | 0.021* | 0.086 | 0.054 |
| 6q | | | 0.028* | 0.078 | |
| 7p | | | 0.045* | 0.068 | 0.094 |
| 7q | 0.046* | -0.013 | | 0.050 | 0.049* |
| 8p | | | 0.073 | 0.070 | 0.035* |
| 8q | | | 0.019* | 0.075 | |
| 9p | | | 0.017* | 0.096 | 0.079 |
| 9q | | | 0.035* | 0.061 | 0.078 |
| 10p | | | 0.020* | 0.082 | 0.069 |
| 10q | 0.033* | -0.016 | 0.008* | 0.092 | |
| 11p | | | 0.074 | 0.065 | |
| 11q | | | 0.045* | 0.087 | |
| 12p | | | 0.079 | 0.056 | 0.017* |
| 12q | | | 0.085 | 0.062 | |
| 13p | | | | 0.031 | 0.043* |
| 13q | | | 0.017* | 0.092 | |
| 14p | | | 0.042* | 0.073 | |
| 14q | | | 0.051 | 0.064 | |
| 15q | | | 0.005* | 0.108 | 0.094 |
| 16p | 0.026* | -0.013 | 0.014* | 0.071 | |
| 16q | | | 0.054 | 0.063 | |
| 17p | | | 0.060 | 0.052 | 0.069 |
| 17q | 0.012* | -0.019 | 0.012* | 0.092 | |
| 18p | | | 0.057 | 0.079 | |
| 18q | | | 0.040* | 0.083 | 0.081 |
| 19p | | | 0.024* | 0.060 | |
| 19q | | | 0.031* | 0.065 | 0.066 |
| 20p | | | 0.013* | 0.091 | |
| 20q | | | 0.070 | 0.055 | 0.023* |
| 21p | | | 0.070 | 0.060 | 0.076 |
| 21q | | | 0.044* | 0.065 | 0.098 |
| 22p | | | | | 0.056 |
| 22q | | | 0.014* | 0.081 | |
| Xp | 0.028* | -0.018 | 0.004* | 0.111 | |
| Xq | | | 0.048* | 0.070 | |

## Table 7: P-values of cognitive function components as predictors of the change in telomere length

| | Cognitive function components | | | | | | |
|---|---|---|---|---|---|---|---|
| | Memory domain | Psychomotor speed domain | Reaction time domain | Complex attention domain | Processing speed domain | Verbal memory domain | Visual memory domain |
| Chromosome | | | | | | | |
| 1p | | | | | | 0.030 | |
| 1q | 0.049 | | | | | 0.016 | |
| 2p | 0.038 | 0.015 | | | 0.045 | 0.015 | |
| 2q | | 0.028 | | | | | |
| 3q | | | | | | 0.015 | |
| 5q | | | | | | 0.019 | |
| 6p | 0.046 | | | 0.042 | 0.017 | 0.019 | |
| 6q | | 0.007 | | | | 0.040 | |
| 7p | 0.049 | | | 0.037 | | 0.028 | |
| 7q | | 0.045 | | | | 0.015 | |
| 8q | | | | | | 0.045 | |
| 9q | | | | | | 0.034 | |
| 10p | | | | | | 0.040 | |
| 10q | | 0.037 | | 0.038 | | 0.029 | |
| 12p | 0.049 | 0.048 | | | | 0.021 | |
| 14p | | | | | | 0.043 | |
| 15q | 0.047 | | | | | 0.013 | |
| 16p | 0.022 | | | 0.019 | 0.030 | 0.028 | 0.038 |
| 16q | | | | | | 0.029 | |
| 17p | 0.049 | | | | | 0.015 | |
| 17q | 0.031 | 0.007 | 0.044 | 0.022 | 0.021 | 0.010 | |
| 19p | | | | | | 0.045 | |
| 19q | | | | | | 0.021 | |
| 20p | | 0.035 | | | | 0.028 | |
| 20q | | | | | | 0.030 | |
| 21p | | | | | | 0.031 | |
| 21q | | | | | | 0.023 | |
| 22q | | | | | | 0.037 | |
| Xp | | 0.019 | | 0.035 | 0.028 | 0.042 | |

**Table 8: Range of p-values of psychoneurological symptoms and non-symptom factors evaluated that showed no significant effect as potential predictors of the change in telomere length**

| Psychoneurological symptom | Range of p-values | Non-symptomatic factors | Range of p-values |
|---|---|---|---|
| Anxiety | 0.357 to 0.983 | Education level | 0.092 to 0.881 |
| Depression | 0.247 to 0.991 | HER2 status | 0.136 to 0.828 |
| Pain | 0.179 to 0.994 | Progesterone receptor status | 0.082 to 0.889 |
| Fatigue | 0.325 to 0.985 | Estrogen receptor status | 0.062 to 0.511 |
| Sleep disturbance | 0.242 to 0.993 | | |

**Association between micronuclei frequency and telomere length**

In an effort to investigate the relationship between micronuclei formation, as an indicator of chromosomal instability, and telomere length attrition we compared micronuclei frequencies and telomere length intensity values using a Pearson correlation test. No significant association was observed between telomere length attrition (indicated by the percent change) and micronuclei frequency [(r ranging from -0.197 to 0.235) and a p-value ranging from 0.268 to 0.979. In addition, we performed a mixed effect model analysis using the raw data, which showed micronuclei frequency was not a significant predictor of the change in telomere length (p-value ranging from 0.091 to 0.996).

# Chapter 4

## Discussion and Future Directions

### Discussion

The purpose of this current work was to assess telomere length in women with early stage breast cancer who were treated with chemotherapy in order to determine if chemotherapy influenced telomere length and if telomere length is related to the development and persistence of PN symptoms. To test our hypothesis that **chemotherapy contributes to chromatin breakage leading to telomere shortening**, we determined chromosome-specific telomere lengths before treatment and at the mid-point of chemotherapy treatment. Consistent with previous studies, our data showed a significant (marginally so in our small sample) negative correlation between age and telomere length. When determining the percent change in telomere length of each chromosomal arm from baseline to mid-chemo, we observed telomere shortening for the majority of chromosomal arms in the majority of study subjects. Several factors may have contributed to this change. First, being triple guanine containing structures, telomeres are highly sensitive to damage by oxidative stress. Therefore, high intensity stress, such as that seen from the cancer itself or its treatment, can accelerate telomere shortening by inducing a high frequency of DNA double strand breaks. Furthermore, oxidative damage contributes to telomere shortening more than that caused by the end

replication problem (reviewed in von Zglinicki, 2002). Thus, since patients with breast cancer have higher levels of oxidative damage than control individuals (Blasiak et al., 2004), the presence (or increase) in reactive oxidative molecules may have contributed to the telomere shortening observed in these patients. Stress also results in activation of the HPA axis, which initiates a cascade of changes involving molecules. One effect of the stress activation pathway is activation of cytokines such as interleukin 2 (IL-2), (IL-6), (IL-8) and (IL-10). Interestingly, chemotherapy has also been associated with higher levels of (IL-6), (IL-8) and/or (IL-10) (reviewed in Seruga et al., 2008). Moreover, the above-mentioned cytokines where found at higher levels in serum samples of patients with breast cancer compared with controls (Lyon et al., 2008). Increased inflammatory activity can accelerate telomere attrition in leukocytes by inducing cell turnover and replicative senescence (Aviv, 2004) or by enhancing the release of reactive oxygen species (ROS) that shortens and damages telomeres through oxidative stress (Jaiswal et al., 2000). A third factor that might induce rapid telomere DNA sequence loss is chemotherapy, which will be discussed in a later part (Li et al., 2012).

While the majority of telomeres showed shortening, some telomeres showed gains in length at the mid-chemo compared to baseline time point. One can speculate that this finding could be attributed to epigenetic alterations, which are known modulators of telomere length (reviewed Blasco, 2007). For example, CCCTC-binding factor (CTCF) is a chromatin insulator protein, which is known to be a negative regulator and an inhibitor of hTERT gene expression. CTCF is regulated by another gene, called brother of the regulator of imprinted sites (BORIS), which codes for a protein that

inhibits CTCF and is detected at high levels in leukocytes of patients with breast cancer compared with controls (D'Arcy et al., 2006). Therefore, by inhibiting the binding of CTCF to hTERT, the activity of BORIS could be epigenetically regulated to influence telomere length.

The use of a chromosome specific approach to assess telomere length allowed us to detect a significant shortening of telomere length on the short arm of chromosome 9 from baseline to mid-chemo. Telomere shortening on the short arm of chromosome 9 could be an early event, as it has been recognized in previous studies of breast cancer risk (Zheng et al., 2009). Telomere dysfunction and/or loss of the short arm of chromosome 9 could lead to increased chromosomal instability for that specific chromosomal arm, which, in turn, could result in an increased susceptibility for chromosome 9 deletions. The short arm of chromosome 9 (9p21) contains a tumor suppressor gene CDKN2A. This gene encodes $p16^{INK4}$ and $p14^{IRF}$ proteins, which are involved in the regulation of the TP53 and retinoblastoma (RB) pathways, the latter of which are considered to play an important role in breast tumor chromosomal instability. Thus, it is feasible that this 9p telomeric attrition is either directly, or indirectly, leading to imbalances that could impact cellular function. We also found a marginally significant decrease involving the telomeres of the long arm of chromosomes 9, 11, 12, 15 and 18 at the mid- chemotherapy time point. While it is likely that the paucity of chromosome-specific telomeres showing attrition is attributable to the small sample evaluated in this study, there is also a possibility that the lack of significant shortening in telomere length

at the mid-chemo time point is due to insufficient toxicity of the treatment to elicit telomeric shortening in the cells.

One important aspect of this study was to investigate the contributory role of telomere shortening to the development and persistence of PN symptoms. By performing correlation studies between telomere shortening in our study participants and the different PN symptoms, we observed a significant positive correlation between telomere shortening and depression. Once again, in many studies of depression, significant increases of oxidative stress (Ng et al., 2008) and inflammation (Dinan, 2008) have been reported. However, the direction of causality in this purported relationship cannot be determined since lymphocytes with shortened telomeres hyper-secrete proinflammatory cytokines, particularly IL-6 (Effros, 2009). While elevated, IL-6 levels in patients having depression have been noted to be comparable in women with or without breast cancer (Musselman et al., 2001). High levels of IL-6 can induce “sickness behavior”, which includes depression as one of its symptoms. However, given that the IL-6 molecule is labile (not retained for long periods of time), its effects may be indirect. While the biological cascade of events is not known, one can speculate that telomere shortening may lead to chromosomal instability or perturbations in gene expression and that these genetic imbalances could then contribute to depressive symptoms that develop in these patients.

In an effort to understand the relationship between telomere shortening and the demographic and behavioral variables measured in this study, we analyzed the ability of these variables to predict the changes seen in telomere length. These tests identified

stress, cognitive function and chemotherapy as predictors of the change in telomere length. Unexpectedly, our mixed effect model suggests that patients with higher scores on the perceived stress scale (PSS) tended to have longer telomere lengths whereas those with higher scores of cognitive function tended to have shorter telomere lengths. The inconsistency of these results with previous studies in the literature could be attributed to our small sample size. Alternatively, the PSS employed in this study may have captured only the most recent stress events, with the length of the telomere being influenced by both current and past stress events. As for the cognitive findings, the metrics that have been used in our study to measure the level of cognitive function have been noted to have some mathematical inconsistencies that may contribute to this observation. Thus, the predictive relationship with cognitive function is considered to be a tentative finding until the questions regarding the measurements have been resolved. In contrast, the observed relationship between chemotherapy and telomere length does not appear to be confounded by methodological factors. Thus, we were able to conclude that chemotherapy is a negative predictor of the change in telomere length (chemotherapy leads to shortening of telomeres). Previous work that has been done by Li and colleagues (2012) strongly supports our observation. Chemotherapeutic agents can contribute to telomere loss by the inhibition of hTERT expression, which in turn reduces telomerase activity. Chemotherapy can also affect telomere length through an effect on telomere-associated proteins (POT1 and TPP1). POT1 binds the single stranded 3’ overhang and suppresses unwanted DNA repair activities. It recruits TPP1, which directly interacts with telomerase for its recruitment to the telomere. Furthermore, TPP1 enhances the affinity of POT1 to the telomeric DNA. Therefore, a reduced

expression of these proteins by chemotherapeutic agents could alter the telomere structure and compromise it's stability, thereby leading to telomere shortening. Doxorubicin (DOX) is a widely used chemotherapeutic drug that is known to interact with the DNA by intercalation and induce DNA double strand breaks (Rossi et al., 2003). Therefore, telomere shortening may also be a consequence of this form of action due to increased cell replication mediated by multiple cycles of injury and repair (Li et al., 2012). Furthermore, DOX may play a major role in telomere shortening by producing free radicals that cause direct oxidative damage to the DNA (Quiles et al., 2002).

The quantification of micronuclei (MN) frequencies in lymphocytes of patients with cancer has been used extensively as a biomarker of chromosomal damage and genomic instability. Indeed, in our study participants, MN frequencies have been shown to be significantly higher than those of controls at baseline levels. In addition, the MN frequencies at the mid-chemo time point are significantly higher than those seen at baseline (Aboalela, 2012). Given that telomeric attrition has been associated with an increased frequency of chromosomal instability and that general chromosomal instability is correlated with MN frequencies, we expected to see a negative correlation between telomere length and micronuclei frequencies (shorter telomeres leading to higher MN levels). However, no clear association was detected. One explanation for this observation is that the sample size is too small to allow for the detection of a significant relationship between these two variables. However, it is also quite feasible that while aberrations related to telomeric attrition following chemotherapy may arise, the majority of the large number of chromosomal changes being induced by chemotherapy may

occur at sites other than the telomere. This latter explanation is consistent with our observation of an increase in MN at mid-chemo, but a lack of association with telomere length change. In conclusion, this study shows that telomere length is shortened after treatment with chemotherapy on the majority of chromosomal arms. Our use of a chromosome specific telomere assay allowed the recognition that this shortening does not affect all telomeres equally, since only a subset of telomeres were most impacted. In particular, the short arm of chromosome 9 was observed to show the greatest attrition response. Furthermore, we showed a negative correlation between telomere length and depression and a negative effect of chemotherapy on the telomere length. While preliminary, these results support our hypothesis that treatment with chemotherapy triggers telomere shortening, which in turn plays a role in the development of PN symptoms.

**Future directions**

This study is designed to assess telomere length in women with breast cancer who are treated with chemotherapy, with the goal of improving our understanding of the role biological events play in the development and persistence of PN symptoms. Data analysis using a mixed effect model to identify predictors of the change in telomere length showed partial inconsistency with the literature. In addition, we noticed an issue with the metrics used for the determination of cognitive function scores. Therefore, it is important to test the reproducibility of our results by increasing the sample size and re-evaluating the accuracy and reliability of the metrics used for the measurement of PN symptoms. Our future studies will involve assessments of more patients (for a total of

75 women), as well as evaluations of additional time points (6 months, 1 year and 2 years post-chemotherapy). By increasing our observations the expanded longitudinal study is expected to provide new insights regarding the relationship between telomere shortening and the acquisition/persistence of PN symptoms in women receiving chemotherapy treatment following their diagnosis of BC. Given that many women are living years after their treatments, this knowledge is important to allow for the recognition of therapeutic and intervention strategies that will be most effective in alleviating their adverse side effects and for ultimately improving their quality of life.

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# Vita

Areej Abdulaziz Alhareeri was born on April 8, 1983, in Riyadh, Saudi Arabia. She graduated from Dar Assalam high school, Riyadh, Saudi Arabia in 2000. She received her Bachelor of Science in Clinical Laboratory Sciences, from King Saud University, Riyadh, Saudi Arabia in 2005 and subsequently worked as a medical technologist in a clinical cytogenetics laboratory in king Abdulaziz Medical City, Riyadh, Saudi Arabia for four years. In 2009, she obtained the American Society Of Clinical Pathology (ASCP) Board of Certification in Cytogenetics. On the same year, Areej received a scholarship from King Saud University for Health Sciences to pursue her graduate study in the field of genetics. She joined the department of Human and Molecular Genetics in Virginia Commonwealth University in 2010.